IN THE DRAWINGS:

A Request for Approval of Drawing Changes is filed concurrently herewith for amendments to Figures 6 and 7.

IN THE SEQUENCE LISTING

Please substitute the paper copy of the Sequence Listing filed with the application for the Sequence Listing filed on June 27, 2001. Please add the computer readable copy of the substitute Sequence Listing filed on June 27, 2000.

REMARKS

Claims 1-31 and 33-63 are pending. Claim 32 has been canceled, claims 1-31, and 33-46 have been amended to conform to proper U.S. patent practice. Claims 47-63 have been added to recite the subject matter deleted from original claims 1-46.

The Specification and drawings have been amended to identify the sequences listed in the substitute Sequence Listing. No new matter has been added.

If there is any fee due in connection with the filing of this Preliminary Amendment, please charge the fee to our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: August 6, 2001

By: Carol L. Cole
Carol L. Cole
Reg. No. 43,555

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APPENDIX TO THE PRELIMINARY AMENDMENT

Prior to the examination of the above application, please amend this application as follows:

IN THE SPECIFICATION:

Please amend the specification as follows:

Please substitute the paragraph located on page 19, lines 30-36, with the following paragraph:

2000 cells transformed with EBV and labeled with ^{51}Cr were incubated for 1 hour in the presence of the indicated hsp70-2 peptides (SEQ ID NOS. 2, 7, 1, and 8) [indicated,] at multiple concentrations. The 11C2 CTL was then added at an effector/target ratio (E/T) of 31/1. Chromium release was measured 4 hours later. The asterisks indicate the mutated amino acids.

Please substitute the paragraph located on page 20, lines 3-12, with the following paragraph:

T2 cells were incubated at 26°C for 16 hours in serum-free medium, with or without peptide at a concentration of 20μm. Next, the peptides (SEQ ID NOS. 2, 7, 1, and 8) were again added, and the cells were incubated at 37°C. At 30-minute or one-hour intervals, the cell pellets were collected and the change in HLA-A2 expression was analyzed by flow cytometry with an anti-HLA-A2 mAb (MA2.1). The amino acid sequences of the peptides are represented[,]. [t]The mutated amino acid is represented by an asterisk.

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Please substitute the paragraph on page 23, lines 2-23, with the following paragraph:

In order to delimit the minimum nucleotide region encoding the antigenic peptide, multiple truncated cDNAs were obtained from the A18 cDNA clone. The use of exonuclease III made it possible to gradually generate deletions starting from the 3' end of the cDNA (Figure 5). These cDNA fragments were cotransfected into COS-7 cells with the autologous HLA-A*0201 cDNA. A minimum coding nucleotide region was located between nucleotides 730 and 944. The truncation in the region carrying the single mutation specific for the tumor abolishes recognition by 11C2 CTLs. Peptides carrying the HLAS-A*0201 binding motif were sought in this region, and among the 18 peptides assayed, only 2 (the nonapeptide SLFEGIDIY (SEQ ID NO: 1), amino acids 286 to 294, and the decapeptide SLFEGIDIYT (SEQ ID NO: 2), amino acids 286 to 295) carrying the mutant isoleucine residue at position 8 were recognized. Maximum half-lysis was obtained with only 5×10^{-11} M of the decapeptide, compared to 5×10^{-7} M of the nonapeptide (Figure 6). 11C2 CTL also recognizes the wild-type decapeptide 286-295 (SLFEGIDFYT) (SEQ ID NO: 7), with a maximum half-lysis of 5×10^{-8} M, but not the wild-type nonapeptide 286-294 (Figure 6).

IN THE CLAIMS:

Please cancel claim 32 without prejudice to or disclaimer of the subject matter contained therein.

Please amend claims 1-31 and 33-46 as follows:

1. (Amended) A method for identifying a peptide [compounds] compound derived from a natural hsp70 sequence, the compound having [which have] at least one

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mutation or [one] modification with respect to the natural hsp70 sequence[, said compounds] and bringing about a T response specific for tumors, comprising [the following steps]:

a) PCR-amplifying a DNA fragment encoding hsp70, obtained from [one or more tumor(s)] at least one tumor,

b) cloning the DNA obtained in step a) into a vector capable of replicating in a bacterium,

c) sequencing [said] a peptide fragment in each bacterial colony obtained after culturing the bacteria of step b), and identifying the [mutation(s)] at least one mutation in hsp70, and

d) determining the immunogenicity of the [mutated] peptide [fragments] fragment having at least one mutation or modification among those identified in step c).

2. (Amended) The method as claimed in claim 1, wherein the immunogenicity of the peptide fragments in step d) [consists of] is determined in an Elispot assay.

3. (Amended) The method as claimed in [either of claims] claim 1 [and 2], wherein the immunogenicity of a peptide [fragments which have] fragment having an anchoring sequence for a given HLA molecule [are preferably] is tested in step d).

4. (Amended) The method as claimed in [one of claims] claim 1 [to 3], wherein the peptide [fragments] to be tested in step d) [are] is obtained by chemical synthesis.

5. (Amended) A method for revealing artificial point mutations or modifications, which can increase the immunogenicity of [the] a mutated peptide [compounds] compound derived from hsp70, [wherein it comprises the following steps] comprising:

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a) determining fragments which have a sequence of [approximately] from 9 to 10 amino acids comprising an anchoring motif for a given HLA molecule,

b) introducing an additional point mutation or modification [(for example a post-translational modification)] at residues chosen from 4, 5, 6, 7 [or] and 8, and

c) determining the immunogenicity of the peptide [fragments] fragment obtained in step b).

6. (Amended) The method as claimed in claim 5, wherein the immunogenicity of the peptide fragment in step c) [consists of] is determined in an Elispot assay.

7. (Amended) A peptide compound [which can be] obtained using a method as claimed in [one of claims] claim 1 [to 5, wherein it comprises] comprising a sequence of at least 8 consecutive amino acids of a natural hsp70 sequence, [which has] the sequence having at least one mutation or [one] modification with respect to the natural hsp70 sequence, and wherein [it] the peptide compound brings about a specific T response.

8. (Amended) The peptide compound as claimed in claim 7, having at least 80% homology with [the] amino acids between positions 286 and 294 of the natural hsp70 sequence.

9. (Amended) The peptide compound as claimed in claim 8, wherein the amino acid at position 293 is [preferably] chosen from isoleucine, leucine, valine, alanine, glycine [or], and phenylalanine[, more particularly isoleucine].

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10. (Amended) The peptide compound as claimed in claim 9, [which has] comprising at least [the] one sequence chosen from SEQ ID No. 1 [or] and SEQ ID No. 2.

11. (Amended) The peptide compound as claimed in [one of claims] claim 7 [to 10], [wherein it comprises] comprising at least one element other than natural amino acids.

12. (Amended) A DNA fragment encoding at least a fragment of the peptide [fragment] compound of [one of claims] claim 7 [to 11].

13. (Amended) A vector for expressing [a] the peptide [fragment] compound as claimed in [one of claims] claim 7 [to 11], [containing the] comprising a DNA fragment encoding a peptide fragment, wherein the DNA fragment is [of claim 12] fused to a promoter which is strong and effective in eukaryotic and/or in prokaryotic cells[, in particular in human cells].

14. (Amended) The [expression] vector as claimed in claim 13, [also] further comprising [one or more] at least one selection [marker(s)] marker and, optionally, [one or more] at least one coding [sequence(s)] sequence for factors which activate [the] immune defenses[, such as cytokines and/or lymphokines].

15. (Amended) The vector as claimed in [either of claims] claim 13 [and 14], wherein [it] the vector is chosen from a viral vector, a plasmid [or], and a pseudovector.

16. (Amended) A dendritic cell loaded with a peptide [compounds] compound as claimed in [one of claims] claim 7 [to 11].

17. (Amended) A dendritic cell transformed with [the expression] a vector as claimed in [one of claims] claim 13 [to 15].

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18. (Amended) The dendritic cell as claimed in [either of claims] claim 16 [and 17], wherein [they belong] the dendritic cell is a macrophage [to macrophages].

19. (Amended) A pharmaceutical composition comprising a peptide compound, or a mixture of peptide compounds, as claimed in [one of claims] claim 7 [to 11] and a pharmaceutically acceptable vehicle.

20. (Amended) The pharmaceutical composition as claimed in claim 19, [wherein it also comprises one or more] further comprising at least one immunological [adjuvants, in particular factors which are cytotoxic for tumors] adjuvant.

21. (Amended) A pharmaceutical composition comprising [an expression] a vector as claimed in [one of claims] claim 13 [to 15] and a pharmaceutically acceptable vehicle.

22. (Amended) A pharmaceutical composition comprising [in particular] a DNA fragment as claimed in claim 12 and a pharmaceutically acceptable vehicle.

23. (Amended) A pharmaceutical composition comprising the dendritic cell [cells] as claimed in [one of claims] claim 16 [to 18] and a pharmaceutically acceptable vehicle.

24. (Amended) A combination product comprising at least one peptide compound as claimed in [one of claims] claim 7 [to 11] and at least one agent which induces cellular stress, for simultaneous or separate use, or for use spread out over time, [intended] for treating cancer.

25. (Amended) The combination product as claimed in claim 24, wherein said at least one agent is capable of inducing overexpression of heat shock proteins[, in particular hsp70].

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26. (Amended) The combination product as claimed in claim 24, wherein said at least one agent is an apoptosis inducer, [selected in particular] chosen from DNA-damaging agents, [and] glucocorticoid receptor ligands, [or from] and pro-apoptotic second messengers.

27. (Amended) The combination product as claimed in claim 24, comprising a viral vector which has a gene which encodes an enzyme for activating pro-apoptotic agents[, in particular thymidine kinase].

28. (Amended) The combination product as claimed in claim 24, [in which] wherein the at least one agent which induces cellular stress is [selected] chosen from compounds which induce tumor hypoxia[, in particular angiogenesis inhibitors].

29. (Amended) The [pharmaceutical composition as claimed in one of claims 19 to 23, or the] combination product as claimed in [one of claims] claim 24 [to 28, wherein it also comprises one or more] further comprising at least one immunological [adjuvants, in particular agents which are cytotoxic for tumors] adjuvant.

30. (Amended) The pharmaceutical composition as claimed in [one of claims] claim 19 [to 23, or the combination product as claimed in one of claims 24 to 29, wherein it comprises] further comprising a pharmaceutical vehicle which is compatible with IV, subcutaneous, oral or nasal administration.

31. (Amended) The pharmaceutical composition as claimed in [one of claims] claim 19 [to 23, or the combination product as claimed in one of claims 24 to 29, wherein it comprises] further comprising a pharmaceutical vehicle [selected] chosen from positively charged liposomes, [or] negatively charged liposomes, nanoparticles, [or] and lipid emulsions.

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33. (Amended) [The use of a peptide compound as claimed in one of claims 7 to 11 for manufacturing a medicinal product intended] A method for treating cancer comprising administering to a patient a medicinal product comprising a peptide compound comprising a sequence of at least 8 consecutive amino acids of a natural hsp 70 sequence, the sequence having at least one mutation or modification with respect to the natural hsp 70 sequence, and wherein the peptide compound brings about a specific T response.

34. (Amended) [The use of a peptide compound as claimed in one of claims 7 to 11 for manufacturing a medicinal product intended for immunization]. A method for immunizing ex situ comprising administering to a patient a medicinal product comprising a peptide compound comprising a sequence of at least 8 consecutive amino acids of a natural hsp 70 sequence the sequence having at least one mutation or modification with respect to the natural hsp 70 sequence, and wherein the peptide compound brings about a specific T response.

35. (Amended) [The use of a peptide compound as claimed in one of claims 7 to 11 for manufacturing a medicinal product intended for immunization] A method for immunizing in situ comprising administering to a patient a medicinal product comprising a peptide compound comprising a sequence of at least 8 consecutive amino acids of a natural hsp 70 sequence the sequence having at least one mutation or modification with respect to the natural hsp 70 sequence, and wherein the peptide compound brings about a specific T response.

36. (Amended) The [use of a peptide compound] method as claimed in [one of claims 7 to 11 for manufacturing a medicinal product intended for treating cancer,

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particularly] claim 33, wherein the cancer is chosen from solid tumors, [especially] carcinomas, melanomas, neuroblastomas, [and] neck cancers, and head cancers[, preferably renal carcinomas].

37. (Amended) [The use of a peptide compound as claimed in one of claims 7 to 11] A method for increasing, in culture medium, [the] a tumor CTL population and/or inducing [the] secretion by said CTLs of cytotoxic factors[, such as for example IL-2, IFN-γ or TNF] comprising providing a peptide compound comprising a sequence of a least 8 consecutive amino acids of a natural hsp 70 sequence the sequence having at least one mutation or modification with respect to the natural hsp 70 sequence, and wherein the peptide compound brings about a specific T response.

38. (Amended) [The use of a peptide compound] A method [as claimed in one of claims 7 to 11 for manufacturing a medicinal product intended] for stimulating [the] immune defenses[, in particular so as to increase the tumor CTL population and/or induce the secretion by said CTLs of cytotoxic factors, such as for example IL-2, IFN-γ or TNF] comprising providing a peptide compound comprising a sequence of at least 8 consecutive amino acids of a natural hsp 70 sequence the sequence having at least one mutation or modification with respect to the natural hsp 70 sequence, and wherein the peptide compound brings about a specific T response.

39. (Amended) The [use] method as claimed in [one of] claim 33 [32 to 38], in combination with radiotheraphy.

40. (Amended) [The use as claimed in one of claim 32 to 39] A method for performing repeated immunization for the purpose of causing a breakdown of tolerance against the corresponding natural peptide (nonmutated) in a patient comprising

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administering to the patient a peptide compound, comprising a sequence of at least 8 consecutive amino acids of a natural hsp 70 sequence, the sequence having at least one mutation or modification with respect to the natural hsp 70 sequence, and wherein the peptide compound brings about a specific T response.

41. (Amended) A method for producing an antibody which binds to an hsp70 mutant[, in particular to the hsp70-2 I-293 mutant,] comprising [the steps consisting in]:

a) immunizing a mammal with a peptide compound [as claimed] in one of claim 7 to 11], comprising a sequence of at least 8 consecutive amino acids of a natural hsp 70 sequence, the sequence having at least one mutation or modification with respect to the natural hsp 70 sequence, and wherein the peptide compound brings about a specific T response and

b) isolating a monoclonal antibody which binds to hsp70-2-293[, particularly to hsp70-2 I-293,] in an immunological assay.

42. (Amended) A monoclonal antibody which binds to a mutated-hsp70 fragment[, particularly to hsp70-2 I-293].

43. (Amended) A method for detecting mutated hsp70[, in particular the hsp70-2 I-293 mutation or modification,] comprising [the steps consisting in]:

a) [bringing] contacting a sample taken from an individual [into contact] with a monoclonal antibody as claimed in claim 42,

b) allowing the formation of [the] an antibody/mutated hsp70 complex[, in particular of the antibody/hsp70-2-293 complex], and

c) detecting mutated hsp70 by means of a detectable label which is in the complex or which binds to the complex.

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44. (Amended) A diagnostic kit <u>for detecting cancer</u> comprising [in particular] an antibody as claimed in claim 42[, for detecting cancer].

45. (Amended) A diagnostic kit <u>for the prognosis of established cancer in an individual</u> comprising [in particular] an antibody as claimed in claim 42[, for the prognostic of established cancer in an individual.

46. (Amended) A pharmaceutical composition comprising [in particular] a monoclonal antibody as claimed in claim 42 and a pharmaceutically acceptable vehicle.

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